

Fig. 2 Western Blot Images showing POI degradation by PROTAC-POI

- A. Images showing POI protein level measured by western blot assay after 24-h treatment with PROTAC-POI at concentrations starting from 3333 nM and 1:3 serial dilution in OCI-LY10 cells.
- B. Degradation Curve of POI. Relative POI level was quantized using Image J software and normalized to both DMSO and cyclophilin A (a loading control). Then, DC50 was determined using the nonlinear regression dose-response equation in GraphPad Prism.

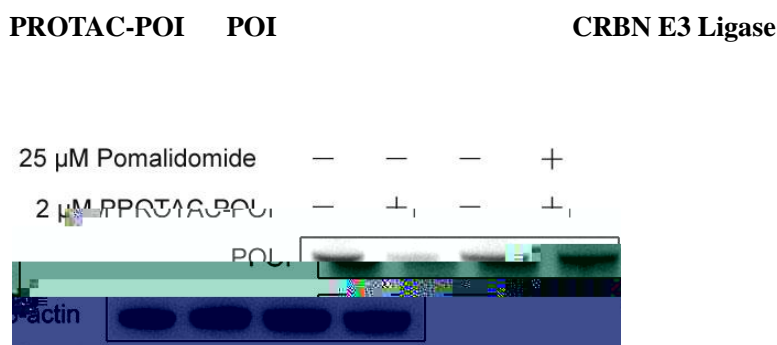


Fig. 3 CUL4-CRBN E3 Ligase mediates PROTAC-POI-induced POI Degradation

CRBN inhibitor pomalidomide for 1 h, then incubated with the combination of PROTAC-POI and pomalidomide for another 24 h. Cells were collected and subjected to western blot assay. β -actin was used as a loading control.

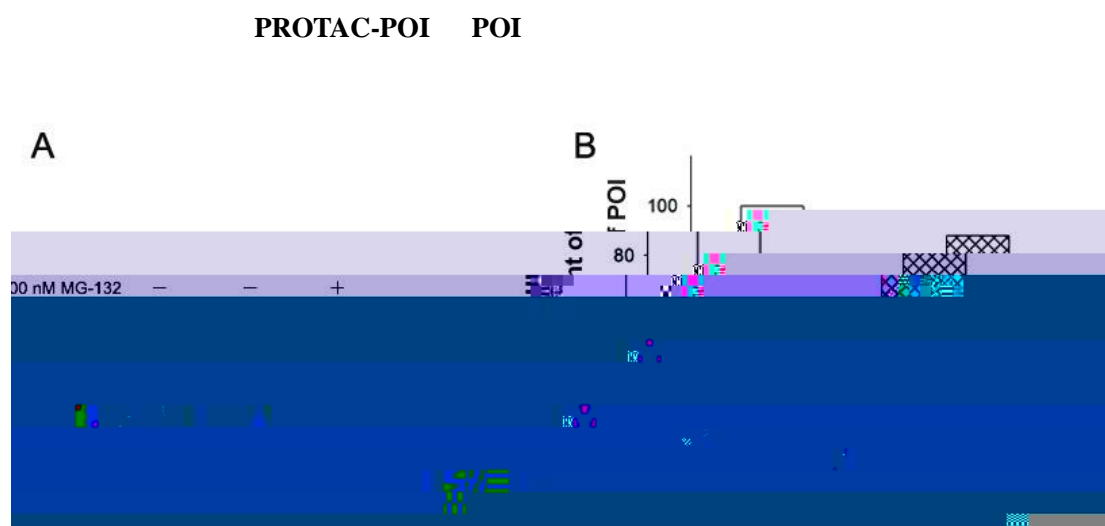


Fig. 4 Proteasome system mediates PROTAC-POI-induced POI Degradation

- A. Western Blot images showing POI level after 1- and then 24-h treatment with PROTAC-POI in HEK 293T cells. β -actin was used as a loading control.



B. Bar chart shows quantized POI level. Protein level in (A) was quantified by using -actin loading control.

PROTAC-POI

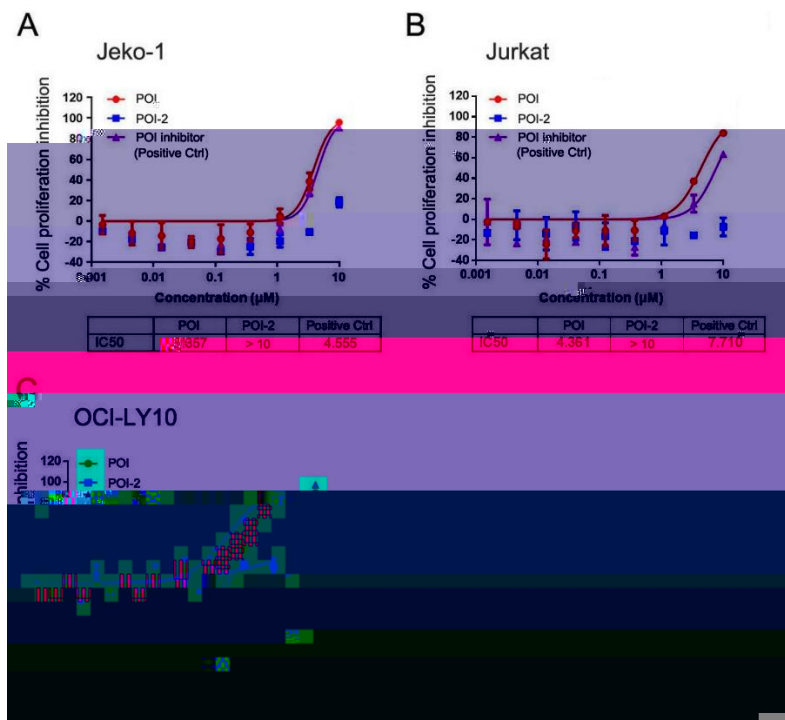


Fig. 5 Proliferation inhibition effects of POI in the indicated cell lines at 120 h after treatment

The cells were treated with the indicated three types of PROTAC molecules for 120 h -fold serial dilution covering 9 different doses. Proliferation inhibition was examined by using the nonlinear regression dose-response equation in GraphPad Prism.

1. Blot POI PROTAC-POI POI Western
2. POI-EGFP PROTAC-POI POI IC50
26 nM; Western Blot PROTAC-POI POI DC50 28.9 nM
HEK293T OE POI-EGFP PROTAC-POI POI
HEK293T OE POI-EGFP PROTAC-POI



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3. CRBN Pomalidomide PROTAC-POI POI
CUL4-DDB1-CRBN E3 PROTAC-POI POI
4. MG-132 PROTAC-POI POI
PROTAC-POI POI
5. PROTAC-POI I